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10/624,909	07/21/2003	Eileen Tozer	564462005300	7087

7590 07/19/2006

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EXAMINER	
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ART UNIT	PAPER NUMBER
1637	

DATE MAILED: 07/19/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/624,909	TOZER ET AL.	
	Examiner	Art Unit	
	Angela Bertagna	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *18 April 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *See Continuation Sheet* is/are pending in the application.

4a) Of the above claim(s) *See Continuation Sheet* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,14,15,29,33,35,40,43-45,48,49,87,188,189,192-207,217-220 and 225-228* is/are rejected.

7)☒ Claim(s) *219 and 220* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *18 April 2006 and 12 July 2003* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date 3/21/2003; 10/20/2004; 2/8/2006; 4/18/2006
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

Continuation of Disposition of Claims: Claims pending in the application are 1,14,15,29,33,35,40,43-45,48,49,51,54,56,58,87,106,107,111,113,116,138,143,174,175,177,182,184,187-190 and 192-228.
Continuation of Disposition of Claims: Claims withdrawn from consideration are 42,51,54,56,58,106,107,111,113,116,138,143,174,175,177,182,184,187,190,208-216 and 221-224.

DETAILED ACTION

Remarks

1. Claims 2-13, 16-28, 30-32, 34, 36-39, 41, 46-47, 50, 52-53, 55, 57, 59-86, 88-105, 108-110, 112, 114-115, 117-137, 139-142, 144-173, 176, 178-181, 183, 185-186, and 191 have been cancelled. Claims 1, 14-15, 29, 33, 35, 40, 42-45, 48-49, 51, 54, 56, 58, 87, 106-107, 111, 113, 116, 138, 143, 174-175, 177, 182, 184, 187-190, and 192-228 are pending. Claims 193-228 are new.

Election/Restrictions

2. Applicant's election with traverse of Group I, claims 1, 14, 15, 29, 33, 35, 40, 43-45, 48-49, 87, 188, 189, and 192, and SEQ ID No: 29 in the reply filed on April 18, 2006 is acknowledged. The traversal is on the ground(s) that Groups III, IV, and XI should be examined with Group I, because these claims, drawn to transgenic nonhuman animals (Group III), transgenic plants and seeds (Group IV), and computer readable media (Group XI), all further comprise the elected SEQ ID No: 29, and therefore a search for SEQ ID No: 29 would necessarily encompass Groups III, IV, and XI. In other words, an undue burden would not be presented by examination of these additional groups with Group I. This is not found persuasive, because the search for a transgenic plant or animal comprising SEQ ID No: 29 requires much more than simply a search for the elected sequence – the only requirement for a search of Group I. The search for transgenic organisms further requires search and examination in the non-overlapping transgenic art, not only for SEQ ID No: 29, but also for evidence of its stable incorporation into an animal, plant or seed. This additional search requirement constitutes a significant examination burden. Also, Group XI, claims 101 and 105, cannot be rejoined with

Group I, because these claims were canceled in the reply filed April 18, 2006. Claims 1, 14-15, 29, 33, 35, 40, 43-45, 48-49, 87, 188-189, 192-207, 217-220, and 225-228 will be examined.

The requirement is still deemed proper and is therefore made FINAL.

Claims 42, 51, 54, 56, 58, 106-107, 111, 113, 116, 138, 143, 174-175, 177, 182, 184, 187, 190, 208-216, and 221-224 and are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on April 18, 2006.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Claim Interpretation

3. Claims 1, 14-15, 29, 33, 35, 40, 43-45, 48-49, 87, 188-189, 192-207, 217-220, and 225-228 recite the phrases "a nucleic acid sequence having" and "a sequence comprising". This language has been interpreted to mean any sequence (dinucleotide or larger) contained in the instant SEQ ID No. 29, and this interpretation is reflected in the application of the prior art below.

Claim Objections

4. Claims 219 and 220 are objected to because of the following informalities: These claims recite non-elected SEQ ID Nos. Appropriate correction is required.

Claim Rejections - 35 USC § 112

5. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1, 14-15, 35, 40, 43-45, 48-49, 87, 188, 193-207, 217-220, and 226-228 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

MPEP notes, "An applicant shows possession of the claimed invention by describing the claimed invention with all of its limitations using such descriptive means as words, structures,

figures, diagrams, and formulas that fully set forth the claimed invention. Lockwood v. American Airlines, Inc., 107 F.3d 1565, 1572, 41 USPQ2d 1961, 1966 (Fed. Cir. 1997)."

In the instant case, the independent claim 1 recites "an isolated, synthetic, or recombinant nucleic acid comprising a nucleic acid sequence having at least 50% sequence identity to SEQ ID No: 29 over a region of at least about 100 residues." SEQ ID No: 29 is 687 nucleotides in length. For a nucleotide sequence of even 6 nucleotides, approximately 27^{20} possible sequences exist with 50% identity. Therefore, for the instant SEQ ID No: 29 with 687 nucleotides, the genus of claim 1 (50% identity over 100 nucleotides) includes an enormous number of sequences, with hundreds of thousands of different molecules. This is a very large genus whose members inherently possess different structural and functional properties.

Regarding genus claims, MPEP notes, "For each claim drawn to a genus:
The written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. See Eli Lilly, 119 F.3d at 1568, 43 USPQ2d at 1406.

"A "representative number of species" means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the

genus. The disclosure of only one species encompassed within a genus adequately describes a claim directed to that genus only if the disclosure "indicates that the patentee has invented species sufficient to constitute the gen[us]." See Enzo Biochem, 323 F.3d at 966, 63 USPQ2d at 1615; Noelle v. Lederman, 355 F.3d 1343, 1350, 69 USPQ2d 1508, 1514 (Fed. Cir. 2004) (Fed. Cir. 2004)("[A] patentee of a biotechnological invention cannot necessarily claim a genus after only describing a limited number of species because there may be unpredictability in the results obtained from species other than those specifically enumerated."). "A patentee will not be deemed to have invented invention of any species other than the one disclosed." In re Curtis, 354 F.3d 1347, 1358, 69 USPQ2d 1274, 1282 (Fed. Cir. 2004)"

Applicant discloses SEQ ID No: 29, which encodes a green fluorescent protein. Applicant further discloses related nucleic acid sequences (for example, SEQ ID Nos: 1-197 (odd SEQ ID Nos: only), but these sequences share a high level of identity (greater than 90%), and therefore do not constitute a representative number of species in the very broad genus outlined above. Furthermore, even within this narrow subgenus, applicant does not demonstrate that all of the members of this subgenus share a common function. The examples on pages 155-159 teach exemplary methods, and the drawings only depict the fluorescence properties of two proteins. Therefore, since applicant only teaches nucleic acid sequences with a very high level of identity to the instant SEQ ID No: 29, with little to no teaching as to their functional properties, and presents no discussion in terms of structural or functional characteristics of sequences with only 50% identity to SEQ ID No: 29, it must be concluded that the requirement to disclose a representative number of species of the broad genus of claim 1 has not been met (see above), and therefore, at the time of filing, applicant did not have possession of the claimed invention.

Claim Rejections - 35 USC § 102

6. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

7. Claims 1, 14, 29, 33, 35, 188-189, and 192-201 are rejected under 35 U.S.C. 102(a) as being anticipated by GenBank Accession No AF401282 (submitted by Lesser et al. August 5, 2001).

Regarding claim 1, GenBank Accession No. AF401282 teaches an isolated nucleic acid sequence comprising at least 50% identity over a region of at least 100 residues to the instant SEQ ID No: 29. See the sequence alignment below, where the instant SEQ ID No: 29 has 70.1% identity to GenBank Accession No. AF401282 over 683 residues.

```
ALIGN calculates a global alignment of two sequences
 version 2.0uPlease cite: Myers and Miller, CABIOS (1989) 4:11-17
seq_29                                                687 nt vs.
gi_15081471_gb_AF401282.1_AF401282 Montastraea fa     683 nt
scoring matrix: DNA, gap penalties: -16/-4
70.1% identity;          Global alignment score: 1488


                    10        20        30        40        50        60
seq_29         ATGAAGGGGGTGAAGGAAGTAATGAAGATCAGTCTGGAGATGGACTGCACTGTTAACGGC
               ::::  : : : ::   ::  :::::::::::  :::   :::::  :: :::: :::::
gi_15081471_   ATGAGTGTGATAAAACCAGACATGAAGATCAAGCTGCGTATGGAAGGCGCTGTAAACGGG
                    10        20        30        40        50        60

                    70        80        90       100       110       120
```

```
seq_29          GACAAATTTAAGATCACTGGGGATGGAACAGGAGAACCTTACGAAGGAACACAGACTTTA
                 :::: ::   :::    :: :: :::: ::  : :::: ::: ::::::::::   :
gi_15081471_    CACAAGTTCGTGATTGAAGGAGACGGAAAGGGCAAGCCTTTCGAGGGAACACAGAGCATG
                       70        80        90       100       110       120

                      130       140       150       160       170       180
seq_29          CATCTTACAGAGAAGGAAGGCAAGCCTCTGACGTTTTCTTTCGATGTATTGACACCAGCA
                 : :::::::  :: ::::::  ::::::: : ::: ::: :::: : :::::: ::: :
gi_15081471_    GACCTTACAGTCAAAGAAGGCGCGCCTCTGCCTTTTGCTTACGATATCTTGACAACAGTA
                      130       140       150       160       170       180

                      190       200       210       220       230       240
seq_29          TTTCAGTATGGAAACCGTACATTCACCAAATACCCAGGCAATATACCAGACTTTTTCAAG
                ::  : :: :: ::: :   :::  ::::::::::    :::::::::::: :::::::
gi_15081471_    TTCGATTACGGCAACAGGGTATTTGCCAAATACCCACAAGATATACCAGACTATTTCAAG
                      190       200       210       220       230       240

                      250       260       270       280       290       300
seq_29          CAGACCGTTTCTGGTGGCGGGTATACCTGGGAGCGAAAAATGACTTATGAGGACGGGGGC
                ::::: :::::  : ::  ::::: :::::::: ::::  :::::::: :: :::  ::::
gi_15081471_    CAGAC-GTTTCCTGAGG--GGTATTCCTGGGAACGAAGCATGACTTACGAAGACCAGGGC
                      250       260       270       280       290

                      310       320       330            340       350
seq_29          ATAAGTAACGTCCGAAGCGACAT--CAGTG-TGAAAGG------TGACTCTTTCTACTAT
                ::  :   :: :  :: ::::::  :: :: ::::::::      ::::: :::   ::::
gi_15081471_    ATTTGCGTCGCCACAAACGACATAACACTGATGAAAGGCGTCGATGACTGTTTTGTCTAT
                300       310       320       330       340       350

                     360       370       380       390       400
seq_29          AAGATTCACTTCACTGGCG---AGTTTCCTCCTCATGGTCCAGTGATGCAGAGAAAGACA
                :: ::::  ::   ::: :   : :::::: :  :::::::::: ::::: :  :::::
gi_15081471_    AAAATTCGATTTGATGGTGTAAACTTTCCTGCCAATGGTCCAGTTATGCAAAAGAAGACG
                360       370       380       390       400       410

               410       420       430       440       450       460
seq_29          GTAAAATGGGAGCCATCCACTGAAGTAATGTATGTTGACGACAAGAGTGACGGTGTGCTG
                 : ::::::::::::::::::::   :::::::::          : :::: :: ::::::
gi_15081471_    CTGAAATGGGAGCCATCCACTGAGAAAATGTATGT---------GCGTGATGGAGTGCTG
                420       430       440       450                 460

               470       480       490       500       510       520
seq_29          AAGGGAGATGTCAACATGGCTCTGTTGCTTAAAGATGGCCGCCATTTGAGAGTTGACTTT
                ::::: ::::: ::::::::::::::::: :::  ::  ::::::   ::  ::::::
gi_15081471_    AAGGGTGATGTTAACATGGCTCTGTTGCTTGAAGGAGGTGGCCATTACCGATGTGACTTC
               470       480       490       500       510       520
```

```
            530        540        550        560        570        580
seq_29       AACACTTCTTACATACCCAAGAAGAAGGTCGAGAATATGCCTGACTACCATTTTATAGAC
             :: ::: : :::: : : ::::::   ::: ::    :::: ::::: :: ::: : :::
gi_15081471_ AAAACTACATACAAAGCTAAGAAGTTTGTCCAGT---TGCCAGACTATCACTTTGTGGAC
            530        540        550        560          570        580

            590        600        610        620          630        640
seq_29       CACCGCATTGAGATTCTGGGCAACCCAGAAGA---CAAGCCGGTCAAGCTGTACGAGTGT
             :: :::::::::::: :: :: ::    ::::   :::   ::: :::::::: :::  :
gi_15081471_ CATCGCATTGAGATTTTGAGCCACGACAAAGATTACAACAAGGTTAAGCTGTATGAGCAT
               590        600        610        620        630        640

               650        660        670        680
seq_29       GCTGTAGCTCGCTATTCTCTGCTGCCTGAGAAGAACAAGTAG
             :: : :::::   :::::  ::::::   : ::  ::::::
gi_15081471_ GCCGAAGCTC---ATTCTGGGCTGCCGAGGCAGGCCAAGTA-
```

Regarding claim 14, the nucleic acid sequence taught by GenBank Accession No. AF401282 encodes a green fluorescent protein (see definition).

Regarding claim 29, GenBank Accession No. AF401282 comprises a sequence that is completely complementary to a sequence shown in SEQ ID No: 29 (see for example, the first three nucleotides of the GenBank sequence "ATG" which are completely complementary to nucleotides 126-128 "TAC" of SEQ ID No: 29 (see above alignment). Note that the phrase "a sequence" has been interpreted to include dinucleotides and larger sequences, and therefore, the GenBank sequence anticipates the instant claim.

Regarding claims 33 and 35, GenBank Accession No. AF401282 teaches a probe comprising at least 10 consecutive bases of a sequence as set forth in SEQ ID No: 29 (see for example, nucleotides 22-31 (ATGAAGATCA) of GenBank Accession No. AF401282 in the above alignment). This was determined by visual inspection.

Regarding claims 188 and 189, GenBank Accession No. AF401282 teaches an isolated nucleic acid sequence encoding a fluorescent protein (see definition) and having at least about 50% identity to SEQ ID No: 29 (see alignment above, where the sequences are 70% identical over 683 nucleotides). As discussed above "a sequence" encompasses dinucleotides or larger, and therefore, GenBank Accession No. AF384683 comprises a sequence as set forth in SEQ ID NO. 29 (for example, the first three nucleotides "ATG").

Regarding claim 192, the sequence recited in GenBank Accession No. AF401282 encodes a fluorescent protein (see definition) and also has a sequence comprising a combination of segments whose overhangs as described in Figure 15 can anneal to each other. Specifically, the GenBank sequence comprises segments with overhangs that can anneal to each other such as GGA which is the "start" overhang in the segment defined by nucleotides 42-44 and the "stop" overhang in the segment defined by nucleotides 98-100 "CCT" (see alignment above).

Regarding claims 193-197, the alignment above between GenBank Accession No. AF401282 and the instant SEQ ID No: 29 displays 70% identity over 683 nucleotides.

Regarding claims 198-201, the alignment between GenBank Accession No. AF401282 and the instant SEQ ID No: 29 displays 70% identity over 683 nucleotides (see alignment above).

8. Claims 1, 15, 29, 33, 35, 40, 43-45, 48-49, 87, 188-189, 192-200, and 225-228 are rejected under 35 U.S.C. 102(b) as being anticipated by Lukyanov et al. (WO 01/27150 A2; cited in IDS).

Regarding claim 1, Lukyanov teaches an isolated nucleic acid sequence (SEQ ID No: 9) comprising at least 50% identity over a region of at least 100 residues to the instant SEQ ID No: 29. See the sequence alignment below, where the instant SEQ ID No: 29 has 60% identity to SEQ ID No: 9 of Lukyanov over 600 nucleotides.

```
ALIGN calculates a global alignment of two sequences
 version 2.0uPlease cite: Myers and Miller, CABIOS (1989) 4:11-17
wipo_seq_9                                          600 nt vs.
seq_29                                              610 nt
scoring matrix: DNA, gap penalties: -16/-4
60.0% identity;            Global alignment score: 747


                  10         20         30         40         50         60
wipo_seq_9    TCAAGGAAGAAATGTTGATCGATCTTCATCTGGAAGGAACGTTCAATGGGCACTACTTTG
              : ::::::: ::::  ::::  :::  :  ::::  : ::  : :: ::  :: : :::
seq_29        TGAAGGAAGTAATGAAGATCAGTCTGGAGATGGACTGCACTGTTAACGGCGACAAATTTA
                  10         20         30         40         50         60

                  70         80         90        100        110
wipo_seq_9    AAATAAAAGGCAAAGGAAAAGGGAAGCCTAATGAAGGCACCAATACCGT-CACGCTCGAG
              : :: :  ::  : :::: :::  : ::: : ::::: ::  : ::  : ::   :  ::
seq_29        AGATCACTGGGGATGGAACAGGAGAACCTTACGAAGGAACACAGACTTTACATCTTACAG
                  70         80         90        100        110        120

             120        130        140        150        160        170
wipo_seq_9    GTTACCAAGGGTGGACCTCTGCCATTTGGTTGGCATATTTTGTGCCCACAATTTCAGTAT
                 :  ::::   : :::::: : :::  ::   :: : :::   :::  ::::::::::
seq_29        AGAAGGAAGGCAAG-CCTCTGACGTTTTCTTTCGATGTATTGACACCAGCATTTCAGTAT
                     130        140        150        160        170

            180        190        200        210        220        230
wipo_seq_9    GGAAACAAGGCATTTGTCCACCACCCTGACGACATACCTGATTATCTAAAGCTGTCA-TT
              ::::::    ::::   : :  :::: : : : ::::: :: : : : : :::: : :  ::
seq_29        GGAAACCGTACATTCACCAAATACCCAGGCAATATACCAGACTTTTTCAAGCAGACCGTT
             180        190        200        210        220        230

              240        250        260        270        280        290
wipo_seq_9    TCCGGAAG-GGATATACATGGGAACGGTCCATGCACTTTGAAGACGGTGGCTTGTGTTGT
              :: ::  : :: ::::: ::::: ::    :::   : ::: ::::: ::: :  ::
seq_29        TCTGGTGGCGGGTATACCTGGGAGCGAAAAATGACTTATGAGGACGGGGGCATAAGTAAC
```

```
            240        250        260        270        280        290

              300        310        320        330        340        350
wipo_seq_9    ATCACCAATGATATCAGTTTGACAGGCAACTGTTTCAACTACGACATCAAGTTCACTGGC
               ::    :  :: :::::: ::: :::  ::: :::: ::::  : ::  : :::::::::
seq_29        GTCCGAAGCGACATCAGTGTGAAAGGTGACTCTTTCTACTATAAGATTCACTTCACTGGC
             300        310        320        330        340        350

              360        370        380        390        400        410
wipo_seq_9    TTGAACTTTCCTCCAAATGGACCCGTTGTGCAGAAGAAGACAACTGGCTGGGAACCGAGC
                :   ::::::::  :::: :: ::  ::::::  ::::::       ::::: ::   :
seq_29        GAG---TTTCCTCCTCATGGTCCAGTGATGCAGAGAAAGACAGTAAAATGGGAGCCATCC
             360          370        380        390        400        410

              420        430                 440        450        460
wipo_seq_9    ACTGAGCGTTTGTATCCTC----------GTGATGGCGTGTTGATAGGAGACATCCATCAT
              :::::      :::::  :           :::: :: ::: :::  :::::  :: :
seq_29        ACTGAAGTAATGTATGTTGACGACAAGAGTGACGGTGTGCTGAAGGGAGATGTCAACATG
               420        430        440        450        460        470

             470        480        490          500        510        520
wipo_seq_9    GCTCTCACAGTGGAAGGAAGGTGGTT--CATTACGTATGTGACATTAA-ACTGTTTACAG
              :::::    :: :    ::: :::    ::::    :  :::: :::: :::  :::::
seq_29        GCTCT----GTTGCTTAAAGATGGCCGCCATTTGAGAGTTGACTTTAACACTTCTTACAT
               480            490        500        510        520        530

                530        540          550        560        570        580
wipo_seq_9    GGCCAAGAAGCCCGTAAAGA---TGCCAGGGTATCACTATGTTGACACCAAACTGGTTAT
               ::::::::   ::  :::   :::: :  :: :: : : : :::  :    : :  ::
seq_29        ACCCAAGAAGAAGGTCGAGAATATGCCTGACTACCATTTTATAGACCACCGCATTGAGAT
                   540        550        560        570        580        590

                   590        600
wipo_seq_9    AAGGAGCAACGACAAAGA
                 : :::::    ::::
seq_29        TCTGGGCAACCCAGAAGA
                   600        610
```

Regarding claim 15, the nucleic acid sequence taught by Lukyanov encodes a cyan fluorescent protein (see Table 1, page 29, where the emission maximum of SEQ ID No: 9

(dsFP483 is reported to be 483 nm. This value is within the emission range for cyan fluorescent proteins).

Regarding claim 29, Lukyanov teaches a sequence that is hybridizes to a sequence completely complementary to a sequence shown in SEQ ID No: 29 (see, for example, nucleotides 3-9 (AAGGAAG) of SEQ ID No: 9 which hybridize to a sequence completely complementary to nucleotides 3-9 (AAGGAAG) fill in of SEQ ID No: 29). Note that the phrase "a sequence" has been interpreted to include dinucleotides and larger sequences, and therefore, the Lukyanov sequence anticipates the instant claim.

Regarding claims 33 and 35, Lukyanov teaches a probe comprising at least 10 consecutive bases of a sequence as set forth in SEQ ID No: 29 (see for example, nucleotides 170-179 in the above alignment: ATTTCAGTAT). This was determined by visual inspection.

Regarding claim 40, Lukyanov teaches an amplification primer pair (see page 12, line 32-page 13, line 4) for amplifying a nucleic acid sequence encoding a polypeptide with fluorescent activity (SEQ ID No: 9 of Lukyanov), where the primer pair is capable of amplifying a nucleic acid comprising a sequence with at least 50% identity to the instant SEQ ID No: 29 (the alignment between SEQ ID No: 9 of Lukaynov & the instant SEQ ID No: 29 is presented above).

Regarding claim 43, Lukaynov teaches an expression cassette comprising the nucleic acid of claim 1 (page 10, lines 12-13).

Regarding claim 44, Lukaynov teaches a vector comprising the nucleic acid of claim 1 (page 10, lines 12-17).

Regarding claim 45, Lukaynov teaches that the vector may be a plasmid, phage, or cosmid (page 2, lines 35-36). Lukaynov also teaches the use of viral vectors, phagemids,

fosmids, bacteriophages, and artificial chromosomes (see page 11, and the cited references therein).

Regarding claims 48 and 49, Lukaynov teaches a transformed cell comprising a vector where the vector comprises a nucleic acid of claim 1 (page 10, line 12 – page 11, line 36).

Regarding claim 87, Lukaynov teaches an array comprising the immobilized nucleic acid of claim 1 (page 13, lines 5-14).

Regarding claims 188 and 189, Lukaynov teaches an isolated nucleic acid sequence encoding a fluorescent protein (see above) and having at least about 50% identity to SEQ ID No: 29 (see alignment below, where the sequences are 52% identical over the full-length SEQ ID No: 29). Also, as discussed above "a sequence" encompasses dinucleotides or larger, and therefore, SEQ ID No: 9 of Lukaynov comprises a sequence as set forth in SEQ ID NO. 29.

```
ALIGN calculates a global alignment of two sequences
 version 2.0uPlease cite: Myers and Miller, CABIOS (1989) 4:11-17
seq_29                                                  687 nt vs.
wipo_seq_9                                              803 nt
scoring matrix: DNA, gap penalties: -16/-4
52.1% identity;            Global alignment score: 263


                                                                    10
seq_29          ATG-----------------------------------------------AAGGGGG
                : :                                               ::: : :
wipo_seq_9      ACGGTCAGGGACACGGTGACCCACTTTGGTATTCTAACAAAATGAGTTGGTCCAAGAGTG
                        10        20        30        40        50        60

                           20        30        40        50        60
seq_29          TGA---AGGAAGTAATGAAGATCAGTCTGGAGATGGACTGCACTGTTAACGGCGACAAAT
                :::   :::::: :::: :::: ::: : :::: : :: : :: :: :: : : :
wipo_seq_9      TGATCAAGGAAGAAATGTTGATCGATCTTCATCTGGAAGGAACGTTCAATGGGCACTACT
                        70        80        90       100       110       120

                 70        80        90       100       110       120
```

```
seq_29          TTAAGATCACTGGGGATGGAACAGGAGAACCTTACGAAGGAACACAGACTTTACATCTTA
                :: : :: :  ::  : :::: :::  : ::: : ::::: ::  : ::  : ::   :
wipo_seq_9      TTGAAATAAAAGGCAAAGGAAAAGGGAAGCCTAATGAAGGCACCAATACCGT-CACGCTC
                       130       140       150       160       170

                130       140       150       160       170       180
seq_29          CAGAGAAGGAAGGCAAG-CCTCTGACGTTTTCTTTCGATGTATTGACACCAGCATTTCAG
                 ::   :  ::::   : :::::: : :::  ::   :: : :::   :::  :::::::
wipo_seq_9      GAGGTTACCAAGGGTGGACCTCTGCCATTTGGTTGGCATATTTTGTGCCCACAATTTCAG
               180       190       200       210       220       230

                 190       200       210       220       230       240
seq_29          TATGGAAACCGTACATTCACCAAATACCCAGGCAATATACCAGACTTTTTCAAGCAGACC
                :::::::::    ::::   : :  :::: : : : ::::: :: : : : :::: : :
wipo_seq_9      TATGGAAACAAGGCATTTGTCCACCACCCTGACGACATACCTGATTATCTAAAGCTGTCA
               240       250       260       270       280       290

                 250       260       270       280       290       300
seq_29          GTTTCTGGTGGCGGGTATACCTGGGAGCGAAAAATGACTTATGAGGACGGGGGCATAAGT
                 :::: ::  : :: ::::: ::::: ::    :::   : ::: ::::: ::: :  ::
wipo_seq_9      -TTTCCGGAAG-GGATATACATGGGAACGGTCCATGCACTTTGAAGACGGTGGCTTGTGT
               300       310       320       330       340       350

                 310       320       330       340       350       360
seq_29          AACGTCCGAAGCGACATCAGTGTGAAAGGTGACTCTTTCTACTATAAGATTCACTTCACT
                    ::   :  :: :::::: ::: :::  ::: :::: ::::  : ::  : ::::::
wipo_seq_9      TGTATCACCAATGATATCAGTTTGACAGGCAACTGTTTCAACTACGACATCAAGTTCACT
                360       370       380       390       400       410

                 370           380       390       400       410       420
seq_29          GGCGAG---TTTCCTCCTCATGGTCCAGTGATGCAGAGAAAGACAGTAAAATGGGAGCCA
                :::  :   ::::::::  :::: :: ::  ::::::  ::::::      ::::: ::
wipo_seq_9      GGCTTGAACTTTCCTCCAAATGGACCCGTTGTGCAGAAGAAGACAACTGGCTGGGAACCG
                420       430       440       450       460       470

                    430       440       450       460       470       480
seq_29          TCCACTGAAGTAATGTATGTTGACGACAAGAGTGACGGTGTGCTGAAGGGAGATGTCAAC
                  ::::::     :::::  :          :::: :: ::: :::  :::::  :: :
wipo_seq_9      AGCACTGAGCGTTTGTATCCTC---------GTGATGGCGTGTTGATAGGAGACATCCAT
                480       490                 500       510       520

                    490           500       510       520       530
seq_29          ATGGCTCT-----GTTGCTTAAAGATGGCCGCCATTTGAGAGTTGACTTTAACACTTCTTA
                   :::::    :: :    ::: :::    ::::    :  :::: :::: :::  :::
wipo_seq_9      CATGCTCTCACAGTGGAAGGAAGGTGGT--TCATTACGTATGTGACATTAA-ACTGTTTA
               530       540       550       560       570       580
```

```
            540        550                 560        570                     580
seq_29        CATACCCAAGAAG-------AAGGT-CGAGAATATGCCT-----GACTACCA------TTT
              ::   ::::::::       ::: : : ::  :::  ::      ::: ::::       ::
wipo_seq_9    CAGGGCCAAGAAGCCCGTAAAGATGCCAGGGTATCACTATGTTGAC-ACCAAACTGGTTA
                590        600        610        620        630        640

                    590        600        610            620            630
seq_29        TATAGACCACCG-CATTGAGATTCTGGGCA----ACCCAGAAGACAAGCC-----GGTCAA
              ::  :: :: :: ::  :: ::::  :  :    :  :  :::  ::::     ::: :
wipo_seq_9    TAAGGAGCAACGACAAAGA-ATTCATGAAAGTTGAAGCTTAAGTAAAGCAAAAAGGTGAC
                650        660        670        680        690        700

                  640             650        660        670        680
seq_29        GCTGTACGA--GTGTGC--TG-TAGCTCGCTATTCTCTGCTGCC-TGAGAAGAACAAGT-
              :  : : ::  :: ::   :: :::   :  ::  :    :: : ::: :  :: :: :
wipo_seq_9    GAGGCATGATAGTATGACATGATAGTATGACATGATAGTATGACATGATAGTAAGAATTG
                710        720        730        740        750        760

seq_29        ------------------------------------AG
                                                   :
wipo_seq_9    TAAGCAAAAGGCTTTGCTTATTAAACTTGTAATTGAAAAC
                770        780        790        800
```

Regarding claim 192, the SEQ ID No: 9 of Lukaynov encodes a fluorescent protein (see above) and also has a sequence comprising a combination of segments whose overhangs as described in Figure 15 can anneal to each other. Specifically, SEQ ID No: 9 of Lukaynov comprises segments with overhangs that can anneal to each other such as GGA which is the "start" overhang in the segment defined by nucleotides 32-34 and the "stop" overhang in the segment defined by nucleotides 135-137 "CCT" (see first alignment above with 60% identity).

Regarding claims 193-197, the alignment above between SEQ ID No: 9 of Lukaynov and the instant SEQ ID No: 29 displays 70% identity over 600 nucleotides (see first alignment presented after claim 1).

Regarding claims 198-200, the alignment below between SEQ ID No: 9 of Lukaynov and the instant SEQ ID No: 29 displays 69% identity over 100 residues (see alignment below). Since the independent claim 1 only requires the identity to be present over a minimum of 100 residues, this alignment meets the instant limitations of claims 198-200.

```
ALIGN calculates a global alignment of two sequences
 version 2.0uPlease cite: Myers and Miller, CABIOS (1989) 4:11-17
seq_29_100_res_320-420                                104 nt vs.
wipo_seq_9_100_res_370-470                            107 nt
scoring matrix: DNA, gap penalties: -16/-4
69.2% identity;            Global alignment score: 226


                    10        20        30        40        50
seq_29_100_r  GACATCAGTGTGAAAGGTGACTCTTTCTACTATAAGATTCACTTCACTGGCGAG---TTT
              :: :::::: ::: :::  ::: :::: ::::  : ::  : :::::::::  :   :::
wipo_seq_9_1  GATATCAGTTTGACAGGCAACTGTTTCAACTACGACATCAAGTTCACTGGCTTGAACTTT
                    10        20        30        40        50        60

               60        70        80        90        100
seq_29_100_r  CCTCCTCATGGTCCAGTGATGCAGAGAAAGACAGTAAAATGGGAGCC
              :::::  :::: :: ::  ::::::  ::::::      ::::: ::
wipo_seq_9_1  CCTCCAAATGGACCCGTTGTGCAGAAGAAGACAACTGGCTGGGAACC
```

Regarding claims 225 and 226, Lukaynov teaches a recombinant nucleic acid encoding a fluorescent protein codon-optimized for expression in a host cell where the nucleic acid comprises a sequence set forth in claim 1 (SEQ ID No: 9 of Lukaynov comprises a sequence set forth in claim 1, as discussed above; see page 14, lines 3-5 and also page 36, lines 1-5 for discussion of codon-optimized forms).

Regarding claim 227, Lukaynov further teaches inclusion of a tag or reporter sequence (page 4, lines 10-12) and also the inclusion of epitope tags (page 9, lines 26-34).

Regarding claim 228, Lukaynov teaches labeled probes (page 12, lines 26-28), and further teaches that nucleic acids may be labeled with epitope tags (page 9, lines 26-34).

9. Claims 1, 14-15, 29, 35, 40, 43-45, 48-49, 188-189, 192, and 198 are rejected under 35 U.S.C. 102(b) as being anticipated by Tsien et al. (USPN 6,140,132).

Regarding claim 1, Tsien teaches an isolated nucleic acid sequence (SEQ ID Nos: 3 and 7) comprising at least 50% identity over a region of at least 100 residues to the instant SEQ ID No: 29. See the sequence alignments below, where the instant SEQ ID No: 29 has 51% identity and 57% identity to SEQ ID Nos: 3 & 7, respectively, of Tsien over 100 nucleotides.

```
ALIGN calculates a global alignment of two sequences
 version 2.0uPlease cite: Myers and Miller, CABIOS (1989) 4:11-17
seq_29                                                 107 nt vs.
tsien_seq_3_egfp                                        86 nt
scoring matrix: DNA, gap penalties: -16/-4
51.4% identity;            Global alignment score: -15


                    10         20         30         40          50
seq_29         GCCTGACTACCATTTTATAGACCACCGCATTGAGATTCTGGGCAA---CCCAGAAGACAA
               ::: ::: :::: :   : :: :::: :: :  :   ::: ::::   :::  : :: ::
tsien_seq_3_   GCCCGACAACCACTACCT-GAGCACC-CAGTCCGCC-CTGAGCAAAGACCCCAACGAGAA
                    10         20         30         40          50

                  60         70         80         90         100
seq_29         GC-CGGTCAAGCTGTACGAGTGTGCTGTAGCTCGCTATTCTCTGCTGCCTG
               :: :: :::    :: :     ::::: :: :::
tsien_seq_3_   GCGCGATCACATGGTCC-----TGCTGGAGTTCG----------------


ALIGN calculates a global alignment of two sequences
 version 2.0uPlease cite: Myers and Miller, CABIOS (1989) 4:11-17
seq_29                                                 107 nt vs.
tsien_cfp_seq_7                                        115 nt
scoring matrix: DNA, gap penalties: -16/-4
57.1% identity;            Global alignment score: 28


                    10         20         30         40          50
seq_29         GCCTGACTACCATTTTATAGACCACCGCATTGAGATTCTGGGCAA---CCCAGAAGACAA
               ::: ::: :::: :   : :: :::: :: :  :   ::: ::::   :::  : :: ::
tsien_cfp_se   GCCCGACAACCACTACCT-GAGCACC-CAGTCCGCC-CTGAGCAAAGACCCCAACGAGAA
```

```
                    10          20          30          40          50

                  60          70                  80          90          100
seq_29          GC-CGGTCAAGCTGTAC-----GAGT--GTG-CTGTAGCTCGCTATTCTCTGCTGCCTG
                :: :: :::     :: :      ::::  ::: : :  ::  :      :::: : :: ::
tsien_cfp_se    GCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCT-CGGCATG
                 60          70          80          90          100         110
```

Regarding claim 14, SEQ ID No: 3 of Tsien encodes a green fluorescent protein (see Table 1, column 5).

Regarding claim 15, SEQ ID No: 7 of Tsien encodes a cyan fluorescent protein (see Table 5, column 5).

Regarding claim 29, Tsien teaches a sequence that is hybridizes under stringent conditions to a sequence completely complementary to a sequence shown in SEQ ID No: 29 (see for example, the first three nucleotides (GCC) of SEQ ID No: 3 of Tsien (in the first alignment above) or the first three nucleotides (GCC) of SEQ ID No: 7 of Tsien (the second alignment above). These sequences hybridize to a sequence completely complementary to nucleotides 1-3 (GCC) of SEQ ID No: 29). Note that the phrase "a sequence" has been interpreted to include dinucleotides and larger sequences, and therefore, the Tsien sequence anticipates the instant claim.

Regarding claim 35, Tsien teaches a probe for identifying a nucleic acid encoding a fluorescent polypeptide, where the probe comprises a sequence of claim 1 (see the above alignments of SEQ ID Nos: 3 & 7 of Tsien). These sequences are inherently probes for a nucleic acid encoding fluorescent polypeptide. This was determined by visual inspection.

Regarding claim 40, Tsien teaches an amplification primer pair for amplifying a nucleic acid encoding a polypeptide with fluorescent activity, where the primer pair is capable of amplifying a nucleic acid comprising a sequence of claim 1 (see column 11, lines 41-46).

Regarding claim 43, Tsien teaches an expression cassette comprising the nucleic acid of claim 1 (column 11, line 57 – column 12, line 40).

Regarding claim 44, Tsien teaches a vector comprising the nucleic acid of claim 1 (column 11, line 66 – column 12, line 40).

Regarding claim 45, Tsien teaches that the vector may be a plasmid, phage, cosmid viral vectors, bacteriophages, and artificial chromosomes (column 13, lines 45-63).

Regarding claims 48 and 49, Tsien teaches a transformed cell comprising a vector where the vector comprises a nucleic acid of claim 1 (column 13, lines 45-63).

Regarding claims 188 and 189, Tsien teaches an isolated nucleic acid sequence encoding a fluorescent protein (see above) and having at least about 50% identity to SEQ ID No: 29 (see alignments below, where SEQ ID Nos: 3 & 7 of Tsien are 49% (about 50%) identical over the full-length SEQ ID No: 29). Also, as discussed above "a sequence" encompasses dinucleotides or larger, and therefore, SEQ ID Nos: 3 & 7 of Tsien comprises a sequence as set forth in SEQ ID NO. 29.

```
ALIGN calculates a global alignment of two sequences
 version 2.0uPlease cite: Myers and Miller, CABIOS (1989) 4:11-17
seq_29                                                  687 nt vs.
tsien_seq_3_egfp                                        720 nt
scoring matrix: DNA, gap penalties: -16/-4
49.4% identity;            Global alignment score: -7

                          10                  20        30        40
seq_29          ATG------AAGGGGGTGAAG---------GAAGTAATGAAGATCAGTCTGGAGATGGAC
                :::      ::::: : : ::         :  ::  ::   :::    : ::: :::::
```

```
tsien_seq_3_  ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGAC
                     10         20         30         40         50         60

                50         60         70         80         90         100
seq_29        TGCACTGTTAACGGCGACAAATTTAAGATCACTGGGGATGGAACAGGAGAACCTTACGAA
               ::   :: :::::: :::  :: :   :  : :: :: ::    :: ::  :   : :
tsien_seq_3_  GGCGACGTAAACGGCCACAGGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTAC
                     70         80         90         100        110        120

              110        120        130        140        150        160
seq_29        GGAACACAGACTTTACATCTTACAGAGAAGGAAGGCAAGCCTCTGACGTTTTCTTTCGAT
              :: :  : :::  :  :  : :     :     ::::::: :   :::   ::  :
tsien_seq_3_  GGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCC--CGTGCCCTGGCCCA
                    130        140        150        160         170

              170        180        190        200        210        220
seq_29        GTATTGACACCAGCATTTCAGTATGGAAACCGTACATTCACCAAATACCCAGGCAATAT-
                 : :  :::: : :  :  :: ::    :     :::: :  ::::: : : : ::
tsien_seq_3_  CCCTCGTGACCACCCTGACC-TACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATG
             180        190        200        210        220        230

                     230        240        250        260        270
seq_29        -ACCAG-----ACTTTTTCAAGCAGACCGTTTCTGGTGGCGGGTATACCTGGGAGCGAAAA
               : :::     :::: ::::::    :: :  : :  :::   ::   :  :::::: :
tsien_seq_3_  AAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGC---TACGTCCAGGAGCGCACC
              240        250        260        270          280        290

            280        290        300        310        320        330
seq_29        ATGACTTATGAGGACGGGGGCATAAGTAACGTCCGAAGCGACATCAGTGTGAAAGGTGAC
              ::    :   ::::::  ::::     ::   :::   :::  : :   :  : :: :::
tsien_seq_3_  ATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGAC
                 300        310        320        330        340        350

            340        350        360         370        380        390
seq_29        TCTTTCTACTATAAGATTCACTTCA--CTGGCGAGTTTCCTCCTCATGGTCCAGTG-----
               :   ::  :    ::  : : ::   :::  : :  ::  : ::: ::   :  :
tsien_seq_3_  AC--CCTGGT-----GAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAAC
                    360            370        380        390        400

                400            410        420        430        440
seq_29        ATGCAGAGAAAGAC------AGTAAAATGGGAGCCATCCACTGAAGTAATGTATGTTGAC-
              :: : : :  : :       :::: ::    : : : ::::  ::    : :::  :: :
tsien_seq_3_  ATCCTGGGGCACAAGCTGGAGTACAACTACAAC-AGCCAC--AACGTCTATATCATGGCC
             410        420        430        440          450        460

              450        460        470        480        490        500
seq_29        GACAAGAGTGACGGTGTGCTGAAGGGAGATGTCAACATGGCTCTGTTGCTTAAAGATGGC
```

```
                ::::::    :    :   : ::::   :  :::: ::    :       :  : :: :::
tsien_seq_3_  GACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGC
                470        480        490        500        510        520

                510        520        530        540        550        560
seq_29        CGCCATTTGAGAGTTGACTTTAACACTTCTTACATACCCAA-GAAGAAGGTC--GAGAAT
                ::    :   :  :::    ::        :::  ::::  :  :: :: :  : :
tsien_seq_3_  AGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTG
                530        540        550        560        570        580

                 570        580        590        600        610        620
seq_29        ATGCCTGACTACCATTTTATAGACCACCGCATTGAGATTCTGGGCAA---CCCAGAAGAC
                :::: ::: :::: :   : :: :::: :: :  :   ::: ::::   :::  : ::
tsien_seq_3_  CTGCCCGACAACCACTACCT-GAGCACC-CAGTCCGCC-CTGAGCAAAGACCCCAACGAG
                590        600        610        620        630        640

                      630             640          650        660
seq_29        AAGC-CGGTCAAGCTGTAC-----GAGT--GTG-CTGTAGCTCGCTAT---TCTCTGC-T
              :::: :: :::    :: :     ::::  ::: : :  :: ::  ::   :::: :: :
tsien_seq_3_  AAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGC-CGGGATCACTCTCGGCAT
                   650        660        670        680        690        700

              670        680
seq_29         GCCTGAGAAGAACAAGTAG
               :   :::  : :::::::
tsien_seq_3_  GGACGAGCTGTACAAGTAA
                    710        720
```

```
ALIGN calculates a global alignment of two sequences
 version 2.0uPlease cite: Myers and Miller, CABIOS (1989) 4:11-17
seq_29                                                  687 nt vs.
tsien_cfp_seq_7                                         720 nt
scoring matrix: DNA, gap penalties: -16/-4
49.0% identity;            Global alignment score: -11
```

```
                          10                  20         30         40
seq_29         ATG------AAGGGGGTGAAG---------GAAGTAATGAAGATCAGTCTGGAGATGGAC
               :::      ::::: : : ::         :  ::  ::   :::    : ::: :::::
tsien_cfp_se   ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGAC
                      10         20         30         40         50         60

                50         60         70         80         90        100
```

```
seq_29          TGCACTGTTAACGGCGACAAATTTAAGATCACTGGGGATGGAACAGGAGAACCTTACGAA
                 ::   :: :::::: :::  :: :   :  : :: :: ::    :: ::  :   : :
tsien_cfp_se    GGCGACGTAAACGGCCACAGGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTAC
                        70         80         90        100        110        120

                  110        120        130        140        150        160
seq_29          GGAACACAGACTTTACATCTTACAGAGAAGGAAGGCAAGCCTCTGACGTTTTCTTTCGAT
                :: :  : :::  :  :  : :     :     ::::::: :  :::   ::  :
tsien_cfp_se    GGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCC--CGTGCCCTGGCCCA
                       130        140        150        160        170

                  170        180        190        200        210        220
seq_29          GTATTGACACCAGCATTTCAGTATGGAAACCGTACATTCACCAAATACCCAGGCAATAT-
                   : :  :::: : :  :     :    : :: : :::: :   ::::: : : : ::
tsien_cfp_se    CCCTCGTGACCACCCTGACCTGGGGCGTGCAGTGC-TTCAGCCGCTACCCCGACCACATG
                180        190        200        210        220        230

                        230        240        250        260        270
seq_29          -ACCAG-----ACTTTTTCAAGCAGACCGTTTCTGGTGGCGGGTATACCTGGGAGCGAAAA
                 : :::     :::: ::::::    :: :  : :  :::   ::   :  :::::: :
tsien_cfp_se    AAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGC---TACGTCCAGGAGCGCACC
                240        250        260        270        280        290

              280        290        300        310        320        330
seq_29          ATGACTTATGAGGACGGGGGCATAAGTAACGTCCGAAGCGACATCAGTGTGAAAGGTGAC
                ::    :  ::::::  ::::     ::   :::   :::  : :   :  : :: :::
tsien_cfp_se    ATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGAC
                   300        310        320        330        340        350

              340        350        360        370        380        390
seq_29          TCTTTCTACTATAAGATTCACTTCA--CTGGCGAGTTTCCTCCTCATGGTCCAGTGATGC
                 :   ::  :    ::  : : ::   :::  : :  ::  : ::: ::   :  :   :
tsien_cfp_se    AC--CCTGGT----GAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAAC
                      360        370        380        390        400

                  400        410        420        430        440
seq_29          AGAGAAAGACAGTAAAATGGGAGCCATCCACTGAAGT---AATGTATGTT-----GACGA
                :      : ::  ::  :::    :: : ::   ::    :: :: : :      : :::
tsien_cfp_se    ATCCTGGGGCAC-AAGCTGGAGTACAACTACATCAGCCACAACGTCTATATCACCGCCGA
                410        420        430        440        450        460

              450        460        470        480        490        500
seq_29          CAAGAGTGACGGTGTGCTGAAGGGAGATGTCAACATGGCTCTGTTGCTTAAAGATGGCCG
                ::::    :    :   : ::::   :  :::: ::    :       :  : :: ::: :
tsien_cfp_se    CAAGCAGAAGAACGGCATCAAGGCCCACTTCAAGATCCGCCACAACATCGAGGACGGCAG
                470        480        490        500        510        520
```

```
            510        520        530        540        550        560
seq_29      CCATTTGAGAGTTGACTTTAACACTTCTTACATACCCAA-GAAGAAGGTC--GAGAATAT
            :     :    :  :::    ::        :::  ::::  :  :: :: :  : :    :
tsien_cfp_se CGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCT
            530        540        550        560        570        580

             570        580        590        600        610          620
seq_29      GCCTGACTACCATTTTATAGACCACCGCATTGAGATTCTGGGCAA---CCCAGAAGACAA
            ::: ::: :::: :   : :: :::: :: :  :   ::: ::::   :::  : :: ::
tsien_cfp_se GCCCGACAACCACTACCT-GAGCACC-CAGTCCGCC-CTGAGCAAAGACCCCAACGAGAA
            590        600        610        620        630        640

                 630             640          650        660           670
seq_29      GC-CGGTCAAGCTGTAC-----GAGT--GTG-CTGTAGCTCGCTAT---TCTCTGC-TGC
            :: :: :::    :: :     ::::  ::: : :  :: ::  ::   :::: :: ::
tsien_cfp_se GCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGC-CGGGATCACTCTCGGCATGG
              650        660        670        680        690        700

                 680
seq_29      CTGAGAAGAACAAGTAG
              :::  : :::::::
tsien_cfp_se ACGAGCTGTACAAGTAA
```

Regarding claim 192, SEQ ID Nos: 3 and 7 of Tsien encode fluorescent proteins (see above) and also have a sequence comprising a combination of segments whose overhangs as described in Figure 15 can anneal to each other. Specifically, SEQ ID Nos: 3 & 7 of Tsien comprises segments with overhangs that can anneal to each other such as GGA which is the "start" overhang in the segment defined by nucleotides 18-20 and the "stop" overhang in the segment defined by nucleotides 116-118 "CCT" (see the alignments above with 49% identity).

Regarding claim 198, the alignment between SEQ ID No: 7 of Tsien and the instant SEQ ID No: 29 displays 57% identity over 100 residues (see alignment appearing after claim 1). Since the independent claim 1 only requires the identity to be present over a minimum of 100 residues, this alignment meets the instant limitations of claim 198.

Claim Rejections - 35 USC § 103

10. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

11. Claims 217 and 218 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lukaynov et al. (WO 01/27150; cited above) in view of Short (WO 00/77262 A1; published December 21, 2000).

Lukaynov teaches a nucleic acid sequence (SEQ ID No: 9) of claim 1, as discussed in greater detail above.

Lukaynov teaches that the nucleic acid may be obtained using non-stochastic site-directed mutagenesis methods (page 13, line 15 – page 14, line 2), but does not teach generation of the recombinant nucleic acid by synthetic ligation reassembly.

Regarding claim 218, Lukaynov teaches expression of recombinant proteins (page 13, line 15 – page 14, line 2).

Short teaches a directed evolution method for evolving nucleic acids encoding novel or improved proteins (see abstract).

Regarding claim 217, Short teaches that standard non-stochastic mutagenesis methods are limited, because only a small number of new, variant products are generated with each application of the method and the types of mutations possible are also limited (see page 4, lines 15-20). Short teaches that synthetic ligation reassembly represents an improvement over these standard non-stochastic site-directed mutagenesis methods, because: (1) it generates a larger number of products with predetermined (non-random) structures with each application; (2) it readily generates more types of mutant polynucleotides, thereby generating a resulting group of mutant products with greater diversity; (3) background resulting from undesired products is decreased; (4) saturation or exhaustive mutagenesis is possible; and (5) the products are produced in a systematic, predetermined fashion (see page 5, lines 1-10).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to utilize the synthetic ligation reassembly method taught by Short to generate recombinant versions of the nucleic acids of Lukaynov. Lukaynov expressly taught production of recombinant polynucleotides using site-directed mutagenesis techniques in order to obtain polynucleotides enoding proteins with improved properties (see page 13, lines 13-31). Since Short taught that synthetic ligation reassembly offered distinct advantages over the conventional methods suggested by Lukaynov, namely the ability to more efficiently and accurately generate a larger number of different, more diverse product sequences (see above), the ordinary practitioner

would have been motivated to utilize this method in order to obtain a faster, simpler method of generating a large variety of mutant polynucleotides.

Conclusion

No claims are currently allowable. Claims 202-207, 219, and 220 are free of the art, but have been rejected for other reasons, as noted above.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. GenBank Accession No. AF384683 (submitted by Lesser et al., Aug. 27, 2001) teaches a sequence highly homologous to the applied GenBank Accession No. AF401282.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Angela Bertagna whose telephone number is (571) 272-8291. The examiner can normally be reached on M-F 7:30-5 pm EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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